MICROBIAL CHARACTERIZATION AND BIODEGRADATION POTENTIAL IN NOVEL FILTRATION SYSTEMS DESIGNED TO REMOVE SULFOLANE FROM POTABLE WATER

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MICROBIAL CHARACTERIZATION AND BIODEGRADATION POTENTIAL IN NOVEL FILTRATION SYSTEMS DESIGNED TO REMOVE SULFOLANE FROM POTABLE WATER

A

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Abstract

Sulfolane is an emerging contaminant used as a solvent in natural gas refineries. In response to a sulfolane spill in North Pole, Alaska, that contaminated portions of the community water supply, a novel water filtration system utilizing granular activated carbon (GAC) was developed and installed in households affected by the contaminated water. While GAC is capable of adsorbing sulfolane, it is unclear whether microorganisms contribute to sulfolane removal in the filtration systems. We characterized the microbial community found within the filtration systems using quantitative PCR (qPCR) and 16S rRNA gene sequencing and assessed the aerobic sulfolane biodegradation potential of the microbial community adhered to the GAC by measuring sulfolane loss over time in incubations using gas chromatography mass spectrometry (GC-MS). Bacterial and archaeal DNA was detected in influent and effluent water as well as throughout the point-of-entry (POE) systems, but on average, influent water contained over 99% more bacterial and archaeal DNA compared to effluent samples. In addition, a difference in microbial biomass was also observed based on the location of the GAC in the filtering system. GAC located distally to the inlet contained more biomass than the GAC that was more proximal within a canister. This difference may be due to increased flow rates at the inlet preventing microbes from adhering to the GAC. The phylum Proteobacteria dominated the bacterial community on the GAC, with over 60% of the sequences assigned to this phylum. No significant biodegradation was observed when GAC used in a POE system was incubated aerobically with sulfolane for 10 weeks. This study provides the first known description of the microbial community in filtration systems used to remove sulfolane. The findings suggest that aerobic microbial processes do not contribute to sulfolane removal in these systems. Instead, processes such as sorption or UV-induced transformations may be responsible for sulfolane removal. Future research on the potential for

anaerobic biodegradation and the production and release of sulfolane breakdown products by anaerobic or photooxidative processes would be warranted.

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Henceforth, we is defined as Sara Janda and Dr. Mary Beth Leigh. We thank Joanna Green and Kristin Hruska for their assistance with DNA extractions, Ian Charold Harriott for his help with qPCR, Michael Willis for his assistance with sulfolane extractions for GC-MS, and Mary-Cathrine Leewis for her help with the statistical analyses and for constructive comments on the manuscript. We also would like to thank Kelly McFarlin, Daniel Pfalmer, Kelly Overduijn, Chris Kasanke, Christa Mulder, Abby Powell, and Corey Oldham for their constructive comments and critiques of this manuscript.

Dedication Page

To my ever-supportive and encouraging fiancé, my driven and inspirational daughter, and my nurturing and thoughtful parents. I love you all.

1. Introduction

Over 300 million tons of synthetic chemicals are used each year worldwide, some portion of which is inevitably released into the environment where it may enter local water supplies (1). Many of these synthetic chemicals can be found in trace amounts throughout the environment and are considered emerging contaminants, which are substances that are often unmonitored yet suspected to cause adverse ecological or human health effects. Unlike persistent organic pollutants and hazardous industrial waste, emerging contaminants are often unregulated due to a lack of research on their health effects, despite their potential threat to the environment (2–4). Emerging contaminants include personal care products, pharmaceuticals, solvents, surfactants, and other compounds used by consumers and industry (2, 5). Chemicals used for industrial activities are especially concerning due to the volume of contaminants that might be accidentally released into the environment.

Sulfolane is a highly polar, non-volatile, odorless solvent commonly used in natural gas and petroleum refineries for separation processes (6, 7). The characteristics that make sulfolane ideal for these processes, such as high water solubility and low affinity for organic matter, also contribute to its persistence in the environment (6, 8, 9). Sulfolane does not sorb to soil when released into the environment; instead, it penetrates directly into the groundwater, allowing it to spread rapidly over a large area and potentially contaminate drinking water. Despite those risks, as an emerging contaminant that has been in use for over 5 decades, sulfolane concentrations in drinking water are often not regulated or monitored and the environmental and human health effects are still not well understood (10). Toxicity studies of sulfolane on animals have shown the potential for negative health effects even at relatively low concentrations, but there is very

limited information available to date on human health effects for either acute or chronic exposure (11).

Current wastewater and drinking water treatments are unable to remove many emerging contaminants, such as sulfolane, from the water supply; therefore, advanced treatment options are often necessary (2, 5, 12). Granular activated carbon (GAC) is of particular interest as a treatment option as it is capable of adsorbing many synthetic organic chemicals as well as natural organic materials, and can be chemically treated to target specific compounds (2, 5, 13). In addition, GAC has also been shown to provide a substrate for microbial growth (14). The presence of biofilms on GAC can significantly enhance the removal of some organic compounds through biodegradation (15, 16). Due to these qualities, GAC is considered to be the "best available technology" for the adsorption of synthetic organic compounds by the US Environmental Protection Agency (USEPA) (17), and thus is commonly used as a treatment option when dealing with emerging contaminants such as sulfolane.

A sulfolane-contaminated groundwater plume was discovered beneath the city of North Pole, Alaska (64°45'4"N, 147°21'7"W) in 2009. In response, the Alaska Department of Environmental Conservation (ADEC) began regulating sulfolane that year, and set a concentration limit of 14 $\mu g\ l^{-1}$ for drinking water (http://dec.alaska.gov/spar/csp/sites/north-pole-refinery/docs/factsheets/develgrndwtrcleanup.pdf). In 2011, the plume was 3.2 by 4 km wide; by 2014, it had expanded to 4 by 4.8 km wide (http://dec.alaska.gov/spar/csp/sites/north-pole-refinery/index.htm). Point-of-entry (POE) filtration systems containing GAC were developed and are currently installed in over 300 private residential wells affected by the contamination (18). GAC has been shown to adsorb sulfolane at water temperatures found in the aquifer and at the rate that water flows through the system (S. C. Janda and D. L. Barnes,

unpublished data). In addition to the GAC, the filtration system also includes other water quality treatments such as sediment filtration and UV radiation. The combined GAC, UV and sediment filtration system was not extensively tested prior to installation, and it is unknown whether microorganisms found in the system may contribute to sulfolane removal through aerobic or anaerobic biodegradation processes. The filtration system is closed and introduces little to no oxygen to the water being filtered, maintaining the anaerobic state of the groundwater. However, there are certain routine procedures in the POE system, such as back-washing, that may introduce oxygen periodically.

Biodegradation of sulfolane by microbial communities has been demonstrated under aerobic conditions with soil matter from a sour gas plant in Canada (8, 10, 19). The addition of nitrogen and phosphorus appeared to stimulate biodegradation at the Canadian plant, though it is not clear whether that soil was nutrient deficient, or if nutrient addition would work at all sulfolane-contaminated sites (10, 19). Anaerobic degradation of sulfolane has not been studied extensively and the results that have been reported are largely inconclusive (6, 8, 9). Anaerobic studies of aquifer and groundwater from the North Pole sulfolane plume have shown no evidence of biodegradation within a year under nitrate- or sulfate-reducing conditions (C. P. Kasanke and M. B. Leigh, unpublished data). There may be certain conditions under which anaerobic sulfolane biodegradation can occur, but the rate is much slower than with aerobic biodegradation (10). It has been demonstrated that the microorganisms present in the sulfolane-contaminated sediment in North Pole, AK are capable of degrading the sulfolane under aerobic conditions, but it is not yet clear if aerobic biodegradation of sulfolane could occur in situ given prevailing redox conditions (C. P. Kasanke and M. B. Leigh, unpublished data).

The goals of our study were to characterize the microbial community structure within the POE systems used in North Pole, and determine if the microorganisms found in the system have the potential to affect sulfolane removal. We present the first description of the bacterial community abundance, structure, and diversity present on GAC and in POE water samples from a sulfolane-contaminated groundwater plume, by using quantitative PCR and 16S rRNA gene amplicon sequencing. We hypothesized that the influent water would have a higher abundance and more diverse community structure compared to the filtered effluent water because of the UV and filtration treatment steps within the POE systems. Our second aim was to determine whether the potential exists for aerobic sulfolane biodegradation by the indigenous microbial communities to occur within the POE systems, using incubation studies and gas chromatography mass spectrometry (GC-MS) analysis to monitor sulfolane concentrations over time. We hypothesized that the microbial community present on the GAC within the POE systems would be capable of biodegrading sulfolane, but not at rates or under conditions sufficient to significantly affect sulfolane concentrations during normal operation of the filtration systems.

2. Methods

2.1 Granular activated carbon sampling

To investigate the microbial community present in spent GAC, we obtained a GAC canister from a POE system on May 15th, 2014 that had treated 78,160 l of sulfolane-contaminated water in North Pole, AK. The canister was stored upon receipt at 4°C and remained capped to prevent contamination. On June 5th, 2014, we divided the canister into thirds (top, middle, and bottom) and took a sample from each section. We placed the GAC samples (500 g) into sterile Nalgene containers and stored them at 4°C.

2.2 Water sample collection

Water samples were collected from 6 POE systems between April and June 2014. Samples were filled directly into sterile 1-l Nalgene bottles with no headspace at a low flow rate to minimize aeration. Samples were immediately placed on ice and transported to the University of Alaska Fairbanks (UAF). We filtered (0.22-µm) the water samples upon receipt, and the filters were stored at -80°C prior to DNA extraction.

To determine how the location within each POE system affected the microbial community structure, we collected water samples from each of the 4 sampling ports (Fig. 1). For every POE system studied, water samples were taken from ports A and D, which correspond to influent and effluent samples, respectively. In addition, ports B and C (representing intermediate treatment steps) were sampled on one of the 6 systems.

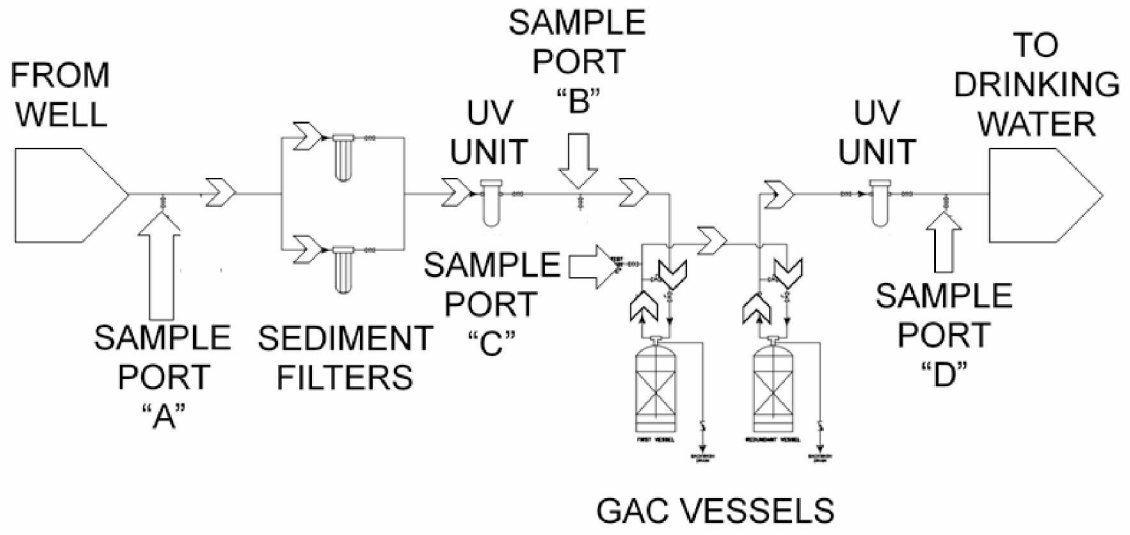

Fig. 1. Diagram of a point-of entry (POE) filtration system used to remove sulfolane from the drinking water in North Pole, Alaska. The system includes 4 sampling ports.

2.3 DNA extraction and analyses

2.3.1 GAC DNA extraction

We isolated DNA from GAC using a PowerMax Soil DNA Isolation Kit (MO BIO, Carlsbad, CA) following the manufacturer's instructions. All samples, including a control consisting of unused GAC, were extracted in duplicate. DNA concentrations were obtained by absorbance at a wavelength of 260 nm with a NanoDrop Lite spectrophotometer (Thermo Fisher Scientific, Waltham, MA). We stored aliquots of the DNA extracts at -20°C.

2.3.2 POE water sample DNA extraction

We used a previously-developed protocol to extract DNA from microbial samples captured on filters (20). Briefly, filters were cut into strips and added to a Lysing Matrix E tube (MP Biomedicals, Solon, OH). Miller lysis and phosphate buffers were added and mixed, followed by a phenol chloroform mixture. Tubes were subjected to bead-beating prior to centrifugation, and the supernatant was removed and mixed with additional chloroform. This solution was centrifuged and the supernatant was mixed with Solution S3 (MOBIO, Carlsbad, CA). All additional extraction and clean up steps were performed according to the instructions in the MOBIO Soil DNA extraction kit (MOBIO, Carlsbad, CA). We stored the final DNA extracts at -20°C.

2.3.3 PCR and qPCR

To determine if the GAC was colonized by microorganisms, we extracted total DNA from GAC and used PCR amplification to test for the presence of bacterial, fungal, and archaeal taxonomic marker genes. To amplify the DNA, we selected universal primers for each domain

(Table 1). For PCR, 10 µl of template DNA, 13 µl of PCR water, and 1 µl (10 µM) of each primer were used with illustra™ puReTaq Ready-To-Go PCR beads (GE Healthcare, Pittsburg, PA). Amplification was carried out on a ProFlex™ 3x32-well PCR system (Life Technologies, Grand Island, NY) with different parameters for each set of primers. For bacterial 16S rRNA genes the PCR parameters were as follows: 1 cycle at 96°C for 5 min, 35 cycles at 95°C for 60 s, 55°C for 60 s, 72°C for 90 s, then 1 cycle at 72°C for 10 min with cooling to 4°C. For the ITS region of fungal rRNA genes the PCR settings were: 1 cycle at 94°C for 3 min, 35 cycles at 94°C for 45 s, 60°C for 45 s, 72°C for 60 s, then 1 cycle at 72°C for 7 min with cooling to 4°C. For archaeal 16S rRNA genes the PCR parameters changed slightly and were as follows: 1 cycle at 94°C for 2 min, 30 cycles at 94°C for 60 s, 55°C for 60 s (for A571F/UA1204R and A751F/UA1406R) or 50°C for 60 s (for A2Fa/U1510R), 72°C for 60 s, then 1 cycle at 72°C for 10 min with cooling to 4°C. Positive and negative controls for each reaction were included.

Table 1. PCR primers used to determine if bacterial, fungal, and/or archaeal DNA was present in spent granular activated carbon (GAC). Due to a lack of agreement on whether current archaeal universal primers can amplify all archaeal DNA, we used three unique universal primer sets for archaeal DNA.

Primer name	**Primer sequence (5'-3')**	**Target**	**Source**
27F 1392R	AGA GTT TGA TCM TGG CTC CGG AAC ATG TGM GGC GGG	Bacterial 16S rRNA gene	(39)
ITS1f ITS2*	CTT GGT CAT TTA GAG GAA GTA A GCT GCG TTC TTC ATC GAT GC	Fungal ITS	(40)
A2Fa U1510R	TTC CGG TTG ATC CYG CCG GA GGT TAC CTT GTT ACG ACT T	Archaeal 16S rRNA gene	(41)
A571F UA1204R	GCY TAA AGS RIC CGT AGC TTM GGG GCA TRC IKA CCT	Archaeal 16S rRNA gene	(42)
A751F UA1406R	CCG ACG GTG AGR GRY GAA ACG GGC GGT GWG TRC AA	Archaeal 16S rRNA gene	(42)
1108F 1132R	ATG GYT GTC GTC AGC TCG TG GGG TTG CGC TCG TTG C	Bacterial 16S rRNA gene (qPCR)	(43)

I = inosine, M = A or C, R = A or G, Y = C or T, W = A or T, and K = G or T

To quantify bacteria and archaea associated with GAC and POE water samples, we performed quantitative real-time PCR (qPCR) on DNA extracts using a synthetic 400-bp dsDNA molecule that contained priming sequence regions from bacterial, archaeal, and fungal gene sequences (Table 2). A dilution series was created using tenfold dilutions down to 0.0004 pM. qPCR was performed on an ABI 7900HT Sequence Detection System (Life Technologies, Grand Island, NY). The master mix consisted of 7.5 µl of SYBR® Green PCR Master Mix (Applied Biosystems, Grand Island, NY), 0.4 µl (10 µM) of each primer, and 3.7 µl of PCR water. The reaction was run using 12 µl of master mix along with 3 µl of template DNA in each well with the following parameters: 1 cycle at 50°C for 2 min, 1 cycle at 95°C for 5 min, 45 cycles at 95°C for 15 s, 60°C for 60 s, and 1 cycle at 95°C for 15 s, 60 °C for 15 s, and 95°C for 15 s. A non-template control (NTC) was also run. We ran all samples, standards, and controls in triplicate. A regression line was created using the standard dilution series and accepted if $R^2 > 0.99$. SDS (version 2.2.2; Life Technologies, Grand Island, NY) was used to analyze the results. There was no preliminary detection of fungi with PCR so it was not included in the qPCR runs.

Table 2. Sequence of synthetic dsDNA fragment and primer sequences using the qPCR assays to quantify bacteria and archaea.

GGTGGTACCA**ttttt**<u>**ATCCATTCAATCGGTACT**</u>**ttttt**<u>**GTGCCAGCAGCCGCGGTAA**</u>**ttttt****CAGCAGCCGC**
GGTAAtttttCAGTTGTACCACGTAGTCTTGAAATCCCACGCAGGTCCAGAAGTACCCGGTATGGTAATA
TAATCTGGTACACGTTGAAACTTCATCATGAACCCGGCCACTCGTTAACCTTTCGACACGGGACACGA
GGCACTGTATCGTATAGTGCGACAAGTCTCCCACTACGGTGTATTGTTGCATTAGTTTCGGGGATTCCG
GCCGATTATAACCGCAGAATACTGCCTATGCTACG**ttttt****ATTAGATACCCTAGTAGTCCttttt****ATTAGAT**
ACCCGTGTAGTCCttttt**CTATTGCTTGCTCTGGAttttt**TAGCACCCC

Fungi: 371bp in vitro; ~390bp in vivo
ATCCATTCAATCGGTAIT = 5'-3' "FR1"
CGATAACGAACGAGACCT = 5'-3' "FF390"

Archaea: 302bp in vitro; ~287 in vivo
CAGCMGCCGCGGTAA = 5'-3' of "A519R" = (519-534)
GGACTACVSGGGTATCTAAT = 5'-3' of "A806R" = (786-806)

Bacteria: 301bp in silico; ~291 in vivo
GTGCCAGCMGCCGCGGTAA = 5'-3' of "533F"
GGACTACNVGGGTWTCTAAT = 5'-3' of "806r

2.3.4 Bacterial community analysis

To assess the bacterial community abundance and diversity in GAC and POE water samples, the V4 region of the 16S rRNA gene was sequenced using a MiSeq (Illumina, San Diego, CA). Primer sets used were based on information from 2 publications (21, 22). We analyzed the raw data using the open-source bioinformatics software mothur (version 1.34; Department of Microbiology & Immunology, University of Michigan [http://www.med.umich.edu/microbio/]) (22). Briefly, both reads for each sample were merged and the sequences were screened to remove those with ambiguous base calls and reduce spurious reads. We then merged all duplicate sequences to reduce the computational burden and aligned them to the SILVA reference database (version 119; http://www.mothur.org/wiki/Silva_reference_files) (23). Following alignment, we re-screened the sequences to ensure all sequences overlapped in the V4 region and to remove any with a homopolymer length greater than 8 base pairs, as no sequences in the reference database have a homopolymer length greater than that. Sequences were filtered to remove gap characters and any overlap outside of the region of interest. We then pre-clustered the sequences, allowing sequences with fewer than 3 nucleotide differences to be merged. Chimeras were identified and removed using the UCHIME algorithm, and any non-bacterial sequences were removed from the dataset as well (24). Sequences were then classified using a Bayesian classifier with the RDP training set (version 10; http://www.mothur.org/wiki/RDP_reference_files) (25).

Rarefaction curves were generated in mothur to determine if there was sufficient coverage of the microbial community, using operational taxonomic units (OTUs) at 97% similarity (22). OTU tables were rarefied to the lowest number of reads for the given sample set (101,000 for GAC samples and 77,000 for POE samples) to correct for uneven sequencing

depths. We computed Good's coverage to assess the completeness of sampling, and the species richness and diversity were determined using the Chao 1 and Inverse Simpson indices (26–28).

The difference in bacterial DNA abundance across the samples was tested with a Student's t-test. Normality was tested prior to running the analysis, and the data were log-transformed if they were non-normal. The t-test was then carried out with the transformed data. The same analysis was used to compare the difference in archaeal DNA abundance across the samples.

Multi-response Permutation Procedures (MRPP), a nonparametric test for differences between 2 or more groups, was conducted to determine if bacterial communities differed among POE systems or between influent and effluent samples. We calculated p-values and effect sizes using a Sørensen distance measure. The effect size, or chance-corrected within-group agreement (A), indicates how similar groups are compared to random chance. A=1 when all samples within a group are identical, and A=0 when the similarity within groups is the amount expected by random chance. Groups were defined by system and by sampling port. There were 6 POE systems studied and an influent (port A) and effluent (port D) sample were collected from each. Additionally, samples were collected from ports B and C in 1 POE system.

We then used Nonmetric Multidimensional Scaling (NMS), an ordination technique commonly used in community ecology, to visualize the microbial community structure across the POE systems. A Sørensen distance measure was used. A single run was completed with real data, following 250 randomized runs to assess the dimensionality of the data. A Monte Carlo randomization test was used to determine whether the final stress could have occurred due to chance alone. To determine the final solution, 25 iterations were run. Stability of the final result was assessed by a stress vs. iteration scree plot. Both the MRPP and NMS analyses were

completed using PC-ORD (version 6; MjM Software) and results were considered significant at $p < 0.05$ (29, 30).

To visually assess how the most abundant OTUs varied among the POE water samples, a heat map was generated using R open-source software (version 3.1.2; Statistics Department, University of Auckland [https://www.stat.auckland.ac.nz/en.html]). We clustered the samples using average-linkage clustering with a Bray-Curtis distance measure.

2.4 Sulfolane biodegradation incubations

To determine if the microbial community on spent GAC was capable of degrading sulfolane aerobically, we performed batch incubation experiments at 4°C using spent GAC incubated with 100 ml of 10-µg l^{-1} sulfolane. An abiotic control (sterilized GAC and 10-µg l^{-1} sulfolane) was used to account for adsorption and evaporation, and a biotic control (spent GAC and sterile water) accounted for desorption of sulfolane from the GAC. The experiments were incubated on a rotary platform shaker (200 r.p.m.) to maintain aerobic conditions. Samples and controls were run in triplicate, and we sampled all experimental flasks and controls immediately after sulfolane addition. Following the initial sampling, 5 ml from each sample and control were removed for analysis by gas chromatography-mass spectrometry (GC-MS) once per week for 10 weeks. Samples were stored at -80°C prior to chemical extraction and analysis.

2.5 Gas chromatography-mass spectrometry (GC-MS)

GC-MS was used to determine sulfolane concentration. Samples were extracted three times in methylene chloride. Sulfolane-d_8 was used as a surrogate to account for extraction efficiency, and nitrobenzene-d_5 was added as an internal standard. Prior to running on the GC-

MS, water was removed from samples using anhydrous sodium sulfate. We ran a continuous calibration verification and laboratory reagent blank on the GC-MS at the start and end of every run, as well as after every 10 samples. Samples were run on an Agilent Technologies 6890 GC system with an Agilent 5973 Mass Selective Detector (Santa Clara, CA). A Restek 30 m RTX-200 column was used with a constant column flow of 1.4-mL min^{-1} and a pulsed-splitless method. The injection volume was 1 µL and injection port temperature was 250°C. Oven temperature was held at 60°C for 1 min, then ramped 10°C min^{-1} to 200°C, then ramped 100°C min^{-1} to 320°C and held for 1 min. The ion range monitored was 40-550 m/z.

2.6 Nucleotide sequence accession numbers

The sequences used in these experiments will be submitted to GenBank (http://www.ncbi.nlm.nih.gov/genbank/) upon publication.

3. Results

3.1 Microbial DNA detection and quantification

We detected bacterial DNA in all GAC and POE water samples analyzed, with the exception of one GAC sample replicate. The abundance of bacterial DNA in GAC ranged from 0 to 0.41 pg μL^{-1} (Fig. 2). Bacterial DNA varied in amount based on the location in the canister ($t(4) = 6.229$, $p < 0.05$). The DNA was more abundant in the top two thirds of the canister compared to the bottom. The abundance of bacterial DNA in POE water samples ranged from 0.02 to 85.1 pg μL^{-1} (Fig. 3). Bacterial DNA was found in higher quantities in the influent water samples as compared to the effluent water samples with the exception of one POE system ($t(4) = 13.74$, $p < 0.05$).

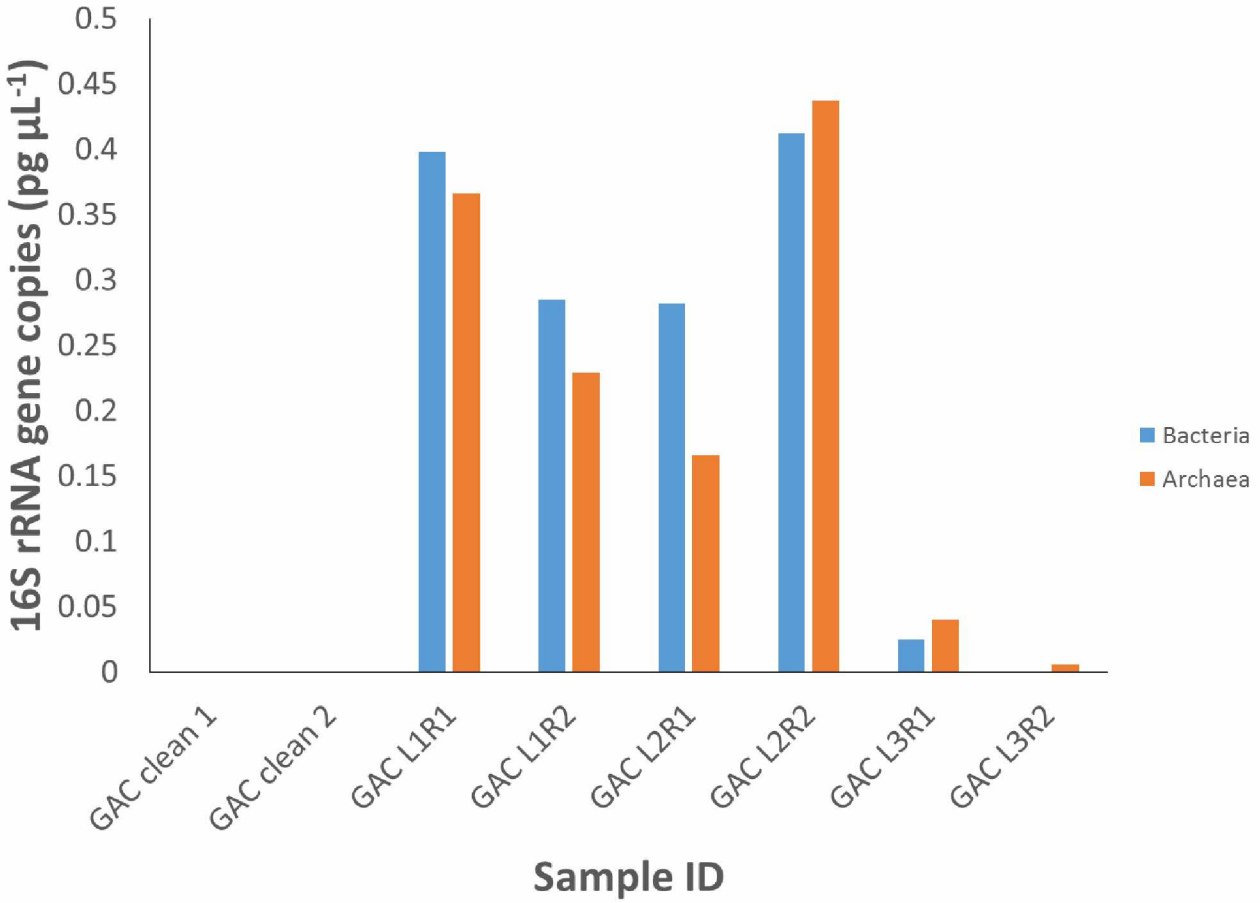

Fig. 2. Absolute abundance of bacterial and archaeal 16S rRNA gene copies in granular activated carbon (GAC) samples (mean of three replicates shown). Clean GAC refers to GAC not yet placed into a point-of-entry (POE) filtration system. All other GAC samples were obtained from a single canister used in a POE system. L1, L2, and L3 refer to the top, middle, and bottom of the canister, respectively. R1 and R2 are replicates 1 and 2. The water inlet on the canisters is in the bottom.

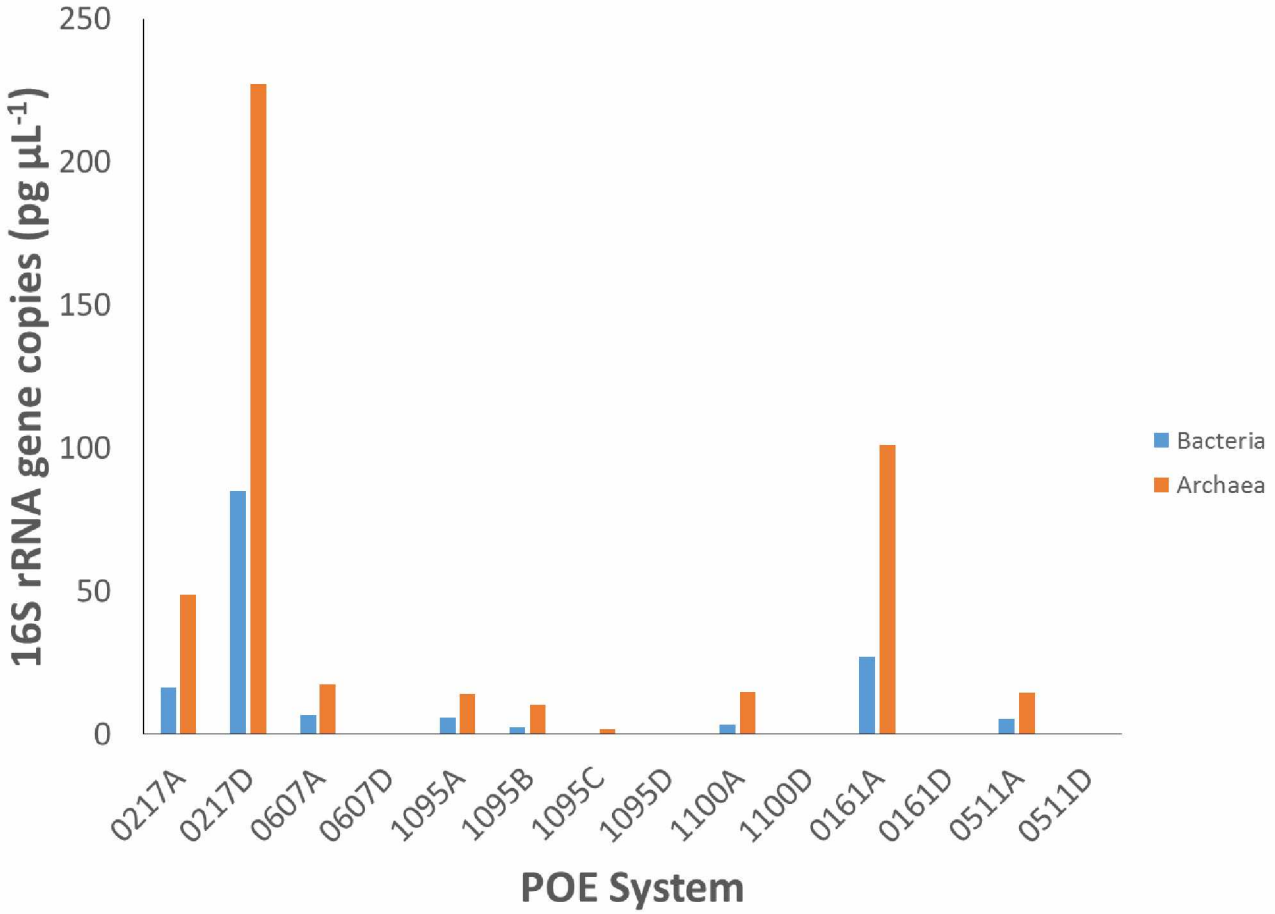

Fig. 3. Absolute abundance of bacterial and archaeal 16S rRNA gene copies in point-of-entry (POE) filtration system water samples (mean of three replicates shown). Sample numbers refer to the POE system identifier, sample letters refer to sampling port location (Fig. 1).

Archaeal DNA was found in all GAC and POE water samples analyzed. The abundance of archaeal DNA in GAC ranged from 0.01 to 0.44 pg μL^{-1} (Fig. 2). Archaeal DNA was also more abundant in the top two thirds of the GAC canister compared to the bottom third ($t(4) = 2.9529$, $p < 0.05$). Archaeal DNA ranged in abundance from 0.03 to 227 pg μL^{-1} in the POE water samples (Fig. 3). Archaeal DNA abundance was higher in the influent samples than in the effluent samples ($t(4) = 11.06$, $p < 0.05$).

3.2 Bacterial community analyses

Following the removal of chimeric and non-bacterial sequences, the number of sequences per GAC sample ranged from 101,226 to 161,005, and from 77,511 to 128,684 per POE water sample. The alpha diversity and richness of the samples varied among POE systems and sampling ports (Table 3). Influent samples were more diverse than effluent samples, with the exception of two of the systems (Fig. 4). Good's coverage ranged from 97-99%, which indicates that 36 to 333 additional reads would have to be processed before encountering a new OTU [1/(1-Good's Coverage)].

Table 3. Alpha diversity estimates and the number of OTUs and sequences after raw sequence processing.

Sample & Port		No. of sequences after processing	Good's coverage	No. of OTUs (97% similarity)	Chao 1	Inverse Simpson
POE Samples	1A	85561	0.992	2621	3356	49.13
	1B	94195	0.991	2701	4027	89.92
	1C	128684	0.996	1236	2515	31.68
	1D	99261	0.993	2564	3425	120.86
	2A	85189	0.995	1561	2136	87.93
	2D	77511	0.996	396	2082	6.01
	3A	83011	0.987	3816	5088	69.18
	3D	78455	0.997	330	2438	6.82
	4A	99632	0.976	6451	8721	74.38
	4D	102854	0.972	8626	11110	176.14
	5A	88560	0.992	2455	3474	110.48
	5D	114152	0.996	754	3329	4.34
	6A	86154	0.978	5095	6962	39.83
	6D	120913	0.997	551	2956	11.24
GAC Samples	GACL1R1	143797	0.995	684	4303	3.16
	GACL1R2	111257	0.996	1136	2166	6.83
	GACL2R1	158051	0.997	484	2518	2.68
	GACL2R2	152092	0.994	794	6473	2.73
	GACL3R1	161005	0.995	639	6026	3.43
	GACL3R2	101226	0.997	516	3126	10.66

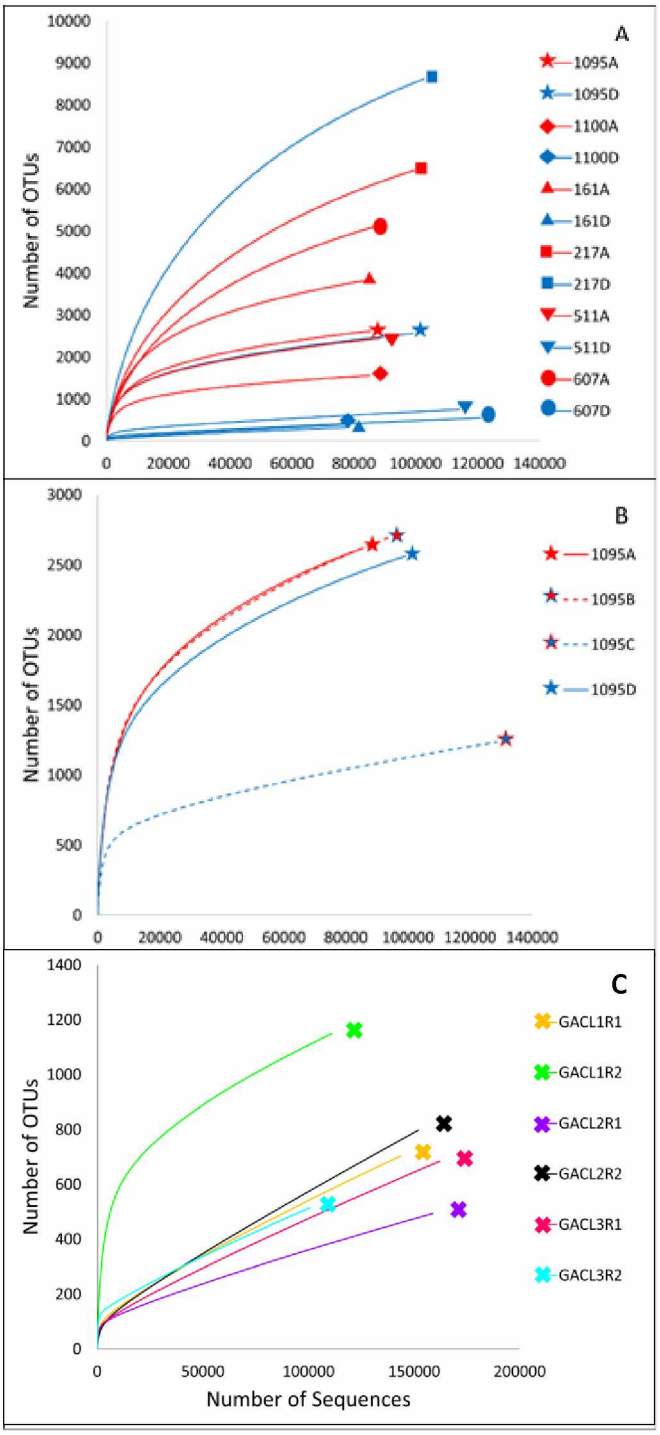

Fig. 4. Rarefaction curves of the 16S rRNA gene sequences for the point-of-entry (POE) filtration systems and the granular activated carbon (GAC). Fig. 4A includes all of the influent and effluent samples from the 6 POE systems sampled; influent samples are red and effluent samples are blue. Different symbols represent different POE systems. Fig. 4B only includes samples from one system, which was sampled at all four sampling ports (A, B, C, and D; Fig. 1). Fig. 4C includes all 6 GAC samples from the GAC canister removed from the same system sampled at all 4 ports. OTUs were defined at 97% similarity.

GAC samples contained 20 classifiable phyla. Over 64% of the sequences were classified in the phylum *Proteobacteria*, with 86% of the *Proteobacteria* classified in the class *Betaproteobacteria*. Unclassified bacteria and the phyla *Firmicutes* and *Actinobacteria* made up 16, 11, and 3% of the community, respectively.

POE water samples contained 24 classifiable phyla. Most communities in POE water samples were dominated by unclassified bacteria, followed by members of the phyla *Proteobacteria, Bacteroidetes,* and *Firmicutes* (Fig. 5). *Betaproteobacteria* and *Deltaproteobacteri*a were the most common classes seen within the phylum *Proteobacteria*.

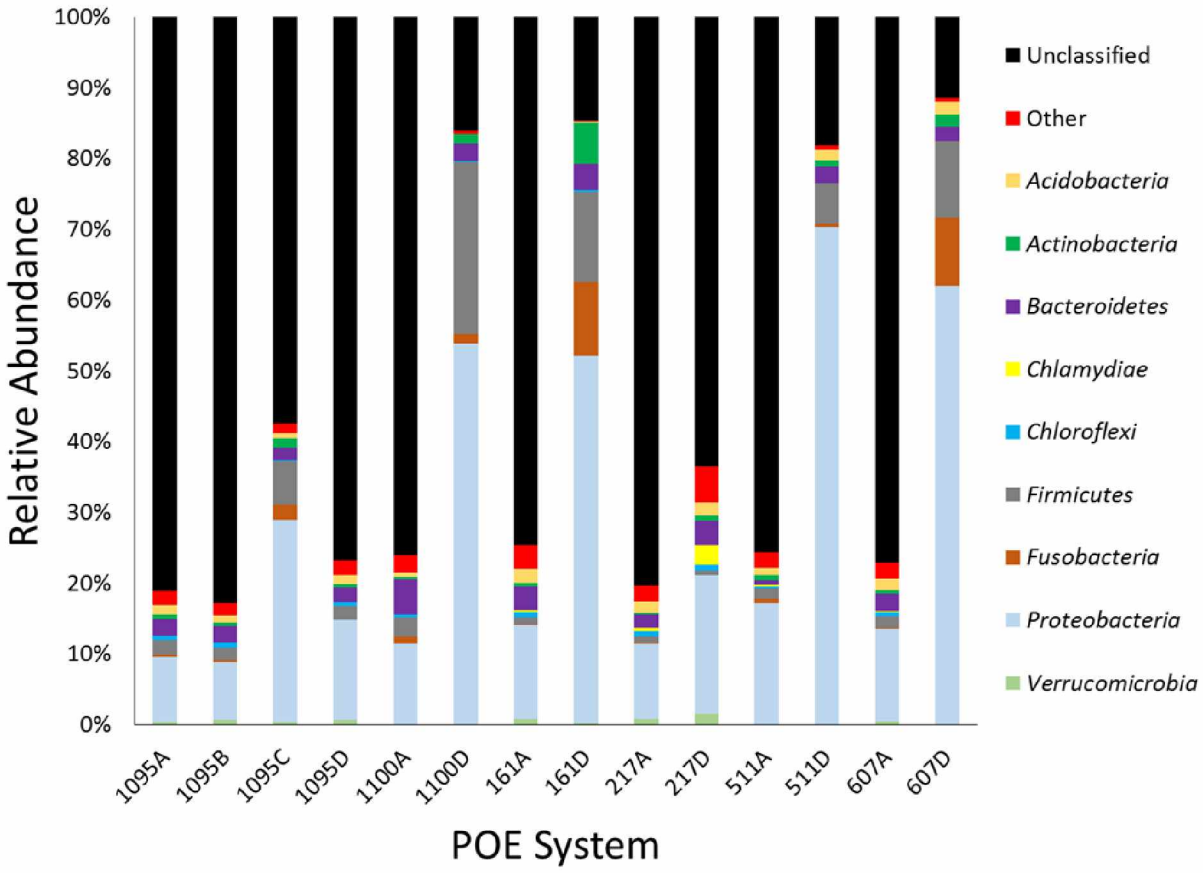

Fig. 5. Relative abundance of bacterial phyla in each sampling location associated with the POE filtration system. Sample numbers refer to the POE system identifier, sample letters refer to sampling port location (Fig. 1). The "other" phylogenetic category includes the phyla Armatimonadetes, Chlorobi, Dienococcus, Gemmatimonadetes, Lentisphaerae, Nitrospira, Planctomycetes, Spirochaetes, Tenericutes, and the proposed phyla BRC1, OD1, OP11, SR1, TM7, and WS3.

The microbial community structures of the influent water samples differed significantly from those in effluent samples (MRPP: A = 0.06, $p < 0.05$; Table 4). An NMS plot was generated to visualize how the communities varied based on presence and relative. Influent water samples formed a distinct grouping towards the positive end of axis 1 and negative end of axis 2, while effluent samples were more distributed and tended to group towards the positive end of axis 2, with two exceptions (stress = 6.95, $r^2 = 0.90$, $p < 0.05$; Fig. 6). Influent (port A) samples tended to cluster together, as did effluent (port D) samples when the presence and relative abundance was compared for the top 200 OTUs (Fig. 7). This suggests that the influent communities from different POE systems were more similar to each other than they were to effluent communities in the same system. Variations in microbial communities were not explained by the POE system of origin or by influent sulfolane concentration (Mantel test: $p > 0.15$).

Table 4. Multi-response permutation procedure (MRPP) test for differentiation of bacterial community structure in POE filtration systems. Comparisons where $p < 0.05$ are shown in bold.

Factor	Effect size, A	Significance, P
POE Port Location	**0.05945**	**0.02199**
Influent vs. Intermediate	0.00487	0.37292
Influent vs. Effluent	**0.06371**	**0.01157**
Effluent vs. Intermediate	0.03304	0.16121

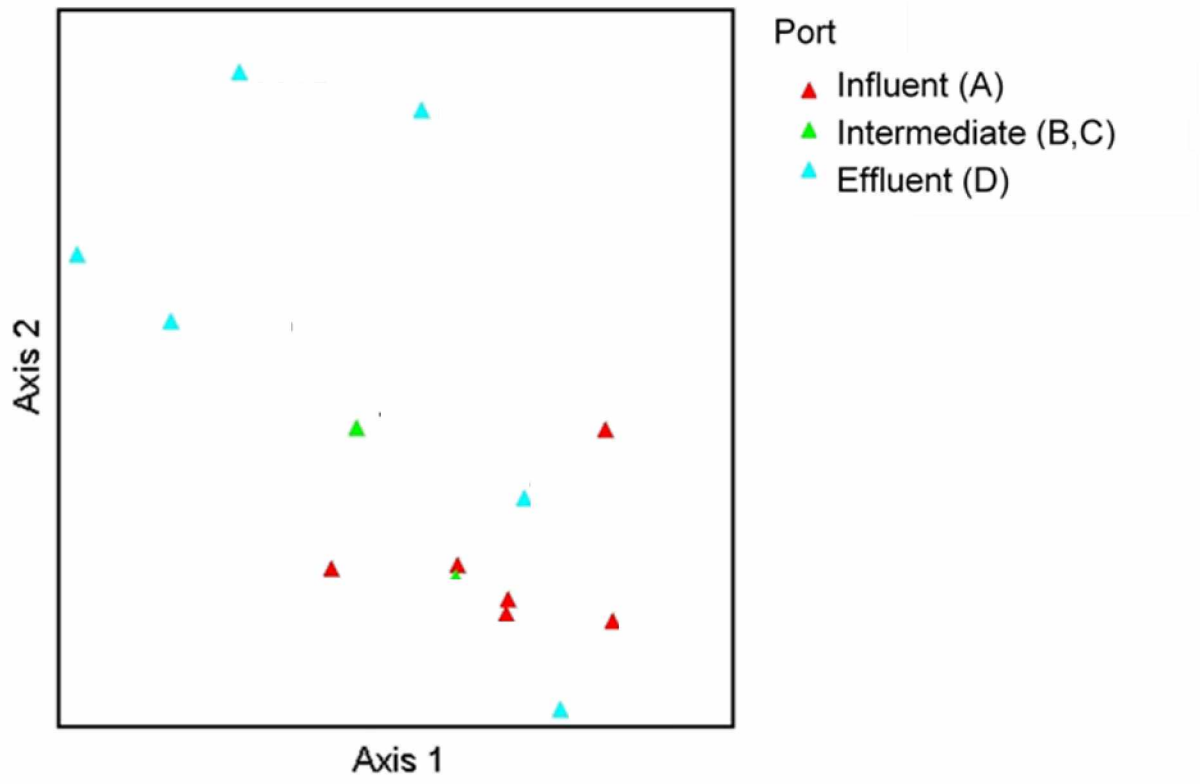

Fig. 6. Nonmetric Multidimensional Scaling (NMS) plot of POE well systems.

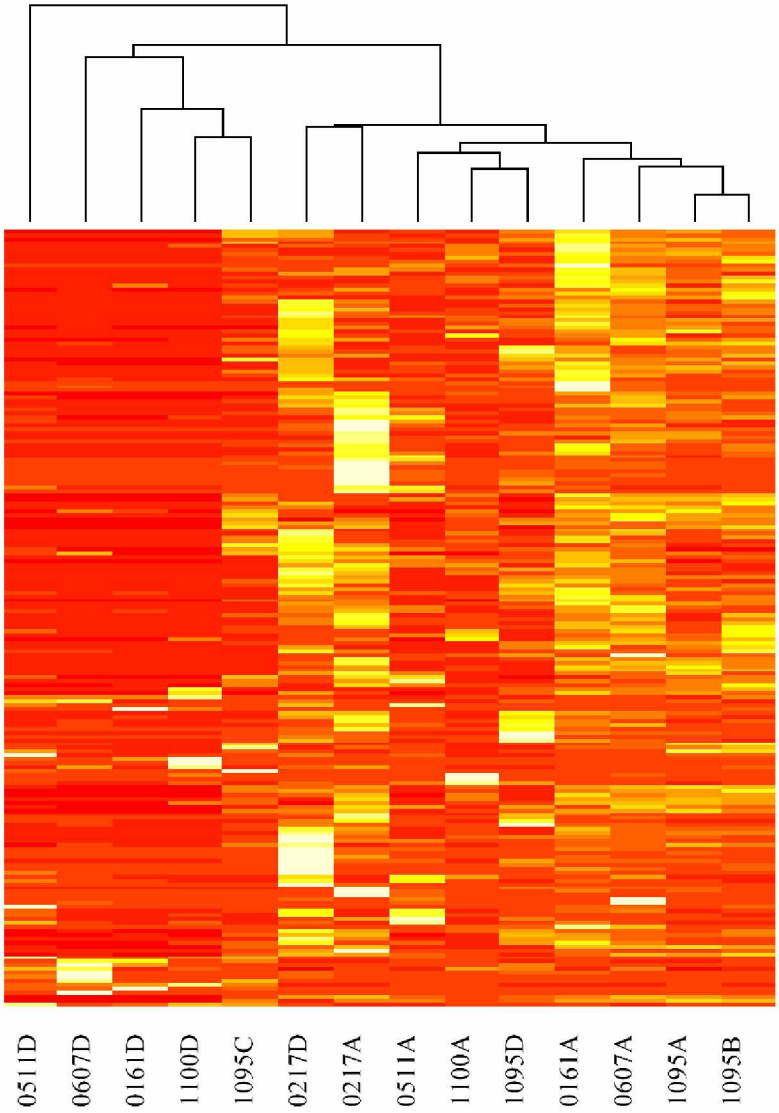

Fig. 7. Heat map describing the abundance and distribution of the top 200 OTUs (defined at 97% similarity) found in POE water samples. Sample numbers refer to the POE system identifier, sample letters refer to sampling port location (Fig. 1). Abundance differences are indicated by color; red indicates low abundance and light yellow indicates high abundance. The dendrogram across the top shows the groupings by sample using complete-linkage clustering with a Euclidian distance measure.

3.3 Sulfolane biodegradation potential

No sulfolane loss was observed during a 10 week aerobic incubation with spent GAC sampled from a POE system. Additionally, we did not see sulfolane loss in the abiotic control, indicating that no evaporation or changes in adsorption occurred, and no sulfolane was detected in the no-sulfolane biotic control, suggesting that sulfolane did not desorb from the GAC.

4. Discussion

We described the diverse bacterial community present in the POE systems used to treat sulfolane in residential wells in interior Alaska. The bacterial community structure varied significantly from influent to effluent and the abundance of bacterial and archaeal DNA was reduced after passing through the POE system, which is likely a result of exposure to UV treatment steps and GAC treatment effects. Additionally, we determined that no significant aerobic biodegradation potential was present in one POE system examined, but understanding whether biodegradation could potentially remove sulfolane under normal operating conditions within this or other POE systems, such as under anaerobic conditions, will require further research.

We determined that the abundance of bacterial and archaeal DNA was, on average, reduced by 99.66 and 99.73% respectively after filtration through the POE system, with the exception of one system (ID 0217). In addition, four of the six POE systems studied showed a dramatic decrease in diversity from influent to effluent samples (Table 2). UV radiation may contribute to the decreases seen, as it has been shown to reduce microbial biomass and alter microbial community structure (31, 32). Additionally, if certain species were more susceptible to UV radiation, this could explain the sudden decrease in diversity seen in these four POE systems. Because our microbial analyses were based exclusively on DNA, we were unable to determine the viability or activity of microbes present.

In two of the six POE systems, bacterial diversity in effluent exceeded that of the influent. One of the systems (ID 0217) also had an increase in abundance of bacterial and archaeal DNA in the effluent water sample. This could be due to differences in system use, with systems that have not had the GAC changed recently harboring more biomass than other

systems, though we were unable to obtain water use data to verify this. Based on data from the POE system sampled at all 4 ports, microbial diversity decreased from ports B to C, indicating that filtration through the GAC affected the community diversity. However, this system also had a higher diversity in the effluent than in the influent, in contrast with most of the other systems studied, and may not be representative of the systems in general. Future studies should focus on a much larger group of POE systems and obtain samples from all ports on each system to further understand how the community structure changes during filtration.

Effluent water and GAC samples contained a larger relative abundance of Proteobacteria than influent water. Proteobacteria are commonly isolated from drinking water and GAC in drinking water filters, and many strains are known to form biofilms (33–36). Biofilms are in constant flux, and can often be sheared from the substrate on which they grew, which may explain the relatively high relative abundance of Proteobacteria in the effluent water samples (14). However, the overall abundance of bacteria in the effluent water was still low compared to influent water samples.

In addition to Proteobacteria, the microbial community associated with GAC contained many other genera with species capable of forming biofilms (Desulfocapsa, Fusobacterium, Streptococcus, etc.). Our study did not determine if a biofilm was actually present on the GAC, though previous research indicates that biofilm formation is likely in similar filtrations systems (14, 37, 38). It is unclear if biofilm formation on the GAC would limit the total amount of sulfolane the GAC was able to adsorb. Future studies should determine if a biofilm is present, and whether it does prevent sorption, which would affect how often the GAC would need to be replaced to maintain its effectiveness.

All GAC and water samples contained high numbers of unclassified bacteria. As bacteria from subarctic aquifers and drinking water have not been extensively characterized, it is possible that there are simply many novel bacteria present in our samples (33). The fact that influent water also contained high proportions of unclassified bacterial DNA suggests that this is not an effect of UV irradiation, since influent water was collected prior to UV exposure.

The microbial community present on spent GAC did not appear to be capable of degrading sulfolane under aerobic conditions over the course of ten weeks. This indicates that microorganisms are unlikely to be contributing appreciably to sulfolane removal within POE systems. Our studies did not investigate the potential for anaerobic biodegradation. There is very limited evidence for anaerobic sulfolane biodegradation in aquifer sediments and groundwater (8, 10, 19). In the presence of certain electron acceptors (e.g. Mn(IV) and NO_3^-), anaerobic biodegradation may occur in some contaminated sites, but the rate is much slower than aerobic biodegradation (10). Therefore, if anaerobic biodegradation potential was present in the POE systems, it would be unlikely to substantially reduce sulfolane concentrations under time scales relevant to operational conditions. Further research on anaerobic biodegradation potential would nonetheless be valuable to confirm if sulfolane biodegradation may occur to any extent in the POE systems, which could be important for the potential production of metabolites.

Our study included only a small sample of POE systems sampled within a limited time frame. To determine if the trends seen in the microbial community structure and abundance are representative of the installed POE systems in this community, a larger sample size and time course will be required, given the heterogeneity observed within this relatively small sample series. While we did not observe any biodegradation potential, further studies are needed to determine if it could occur *in situ* under operational conditions. If biodegradation does occur in

the systems, microorganisms may potentially be utilized to more efficiently reduce sulfolane concentrations, but attention should be given to the potential for metabolite production.

Our study characterized the microbial community present in filtration systems used to remove sulfolane from potable water and helped to clarify the potential role of microorganisms in these systems. We found a diverse microbial population in the contaminated groundwater and determined that filtration through the POE system reduced bacterial and archaeal abundance and often, but not always, reduced diversity within the treated drinking water. Because microbial detection was based on DNA, we were unable to determine the viability or activity of the microbes present. We found no evidence for aerobic biodegradation potential within a 10-week batch incubation study, which is far in excess of the typical residence time of water within POE systems. The potential for anaerobic biodegradation to reduce sulfolane concentrations in these POE systems remains unknown, but if present, is unlikely to appreciably reduce sulfolane concentrations within a time period relevant to system operation. The findings suggest that, while bacterial and archaeal DNA is present, microbial processes are not playing a major role in sulfolane removal in POE systems. Rather, processes like adsorption and potentially UV-induced degradation or transformation are likely responsible for sulfolane removal, and warrant further investigation.

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